安田女子大学紀要　37，345–357　2009.

The Possible Positions of Amino Acid Side Chains in Ion Channel Antibiotic Gramicidin A: On the Base of the β-Helical Polypeptide Backbone Structures by X-ray Structure Analysis

Michio KIMURA

イオンチャンネル抗生物質であるグラミシジン A のアミノ酸側鎖立体構造 ——X 線構造解析で得られたグラミシジン A 構造の β-Helcal Polipeptide Backbone を基にして——

木　邑　道　夫

The author has been studying the structure of ion channel gramicidin A in membranes. This paper considers the movement in membranes of side chains for the structure of gramicidin A amino acid sequence. The backbone of a polypeptide structure can rather severely limit the steric range allowed conformations for large, relatively inflexible side chains, such as the indole ring of tryptophan. This paper describes a method for determining the possible locations of side chains in a polypeptide helix, by taking into account the steric requirements. First, the method attaches side chains to the backbone using the concept of least-squares best molecular fit to the crystal structures of single amino acids. The side chains are then rotated about the Cα-Cβ and the Cβ-Cγ bonds, to select those conformations which exhibit no unfavorable steric contacts with the polypeptide backbone. The method has been applied to the attachment of four tryptophan side chains to a proposed β-helical backbone model of gramicidin A.

Key words : tryptophan, β-helical pepetides, ion-channels, antibiotics, polypeptides

I. Introduction

Gramicidin A is a linear pentadecapeptide antibiotic from *Bacillus brevis* that facilitates the diffusion of monovalent cations (K^+, Cs^+) across membranes[1] by forming transmembrane channels, each of which is made up of two molecules (dimer) of gramicidin A (Fig. 1a, 1b). As suggested by Urry[2] the gramicidin channels are dimers of $\beta^{6.3}$-helical monomers that are joined by six hydrogen bonds at their formyl-NH termini (Fig. 1). The amino acid seguence of valine gramicidin A is altenative structure;

HCO(formyl)-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp(ZZZ)-D-Leu-L-Trp-D-Leu-L-Trp-NHCH$_2$CH$_2$OH(ethanolamine)

where ZZZ is tryptophan in gramicidin A, phenylalanine in gramicidin B, and tyrosine in grami-

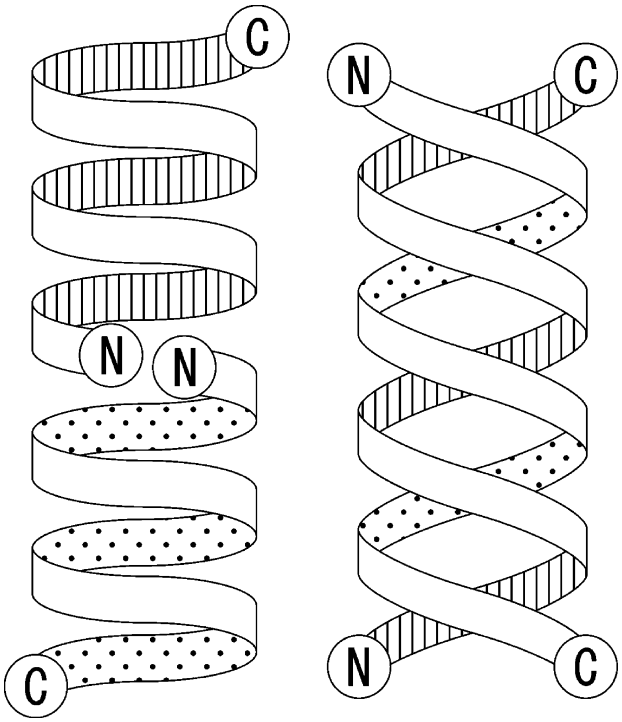

Fig. 1a Schematic diagrams of the gramicidin A described in the text, the channel (helical dimmer) and pore (double helix) conformations. (ref. 3)

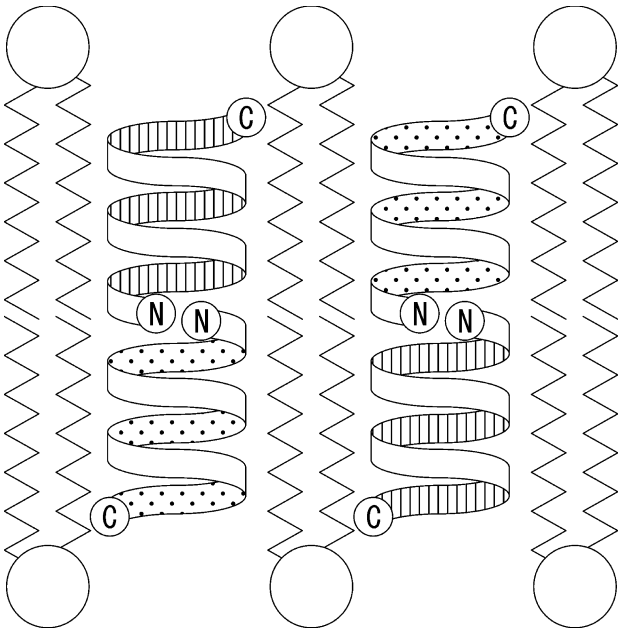

Fig. 1b Models proposed for the conformation of the gramicidin A trnsmembrane channels.

cidine C. Gramicidin D is the name given to the natural mixture of garamicidins A, B, and C, which are present in an approximate ratio 80 : 5 : 15.[3] Tow established mechanisms of achieving ion flux across lipid bilayer membranes are by neutral carriers[2] such as valinomycin and nonactin in Fig. 2a, 2b, and by the formation of transmembrane structrures as occur with nystatin (Fungicidin), amphotericin B (Fungizone), tyrocidine in Fig. 3a, 3b and 3c and ion channel gramicidin A. The two possible types conformations of gramicidin A have been proposed in 1987 the channel and pore structures (Fig. 1a), which correspond to the forms primarily found in membranes and in organic solutions, respectivel. Models were proposed for the conformation of the gramicidin transmembrane channel made up of two molecules (Fig. 1b).[1] A general method is presented for computing the atomic coordinates of helices in which a dipeptide is the repeating unit.[4] Antiparallel dimmer of single-stranded β-helices (P=4.85, N=6.3) have been presented.[4]

In the basis of a solved crystal structure, one must resort to less direct methods for information regarding the tertiary structure of a protein in biological membranes. Spectroscopic techniques

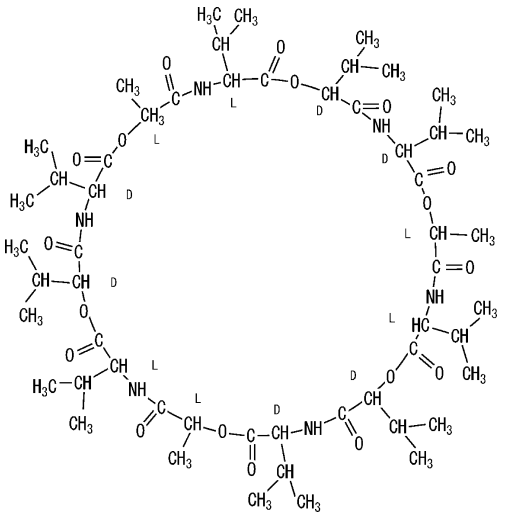

Fig. 2a Valinomycin
Cyclododecadepsipeptide antibiotic produced by *Streptomyces fulvissimus*.

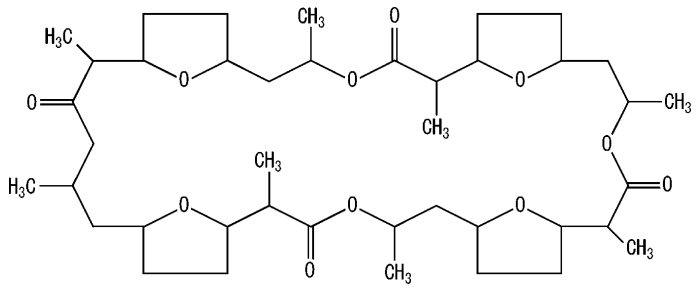

Fig. 2b Nonactin
Macrotetrolide antibiotic produced by several *Streptomyces* spp.

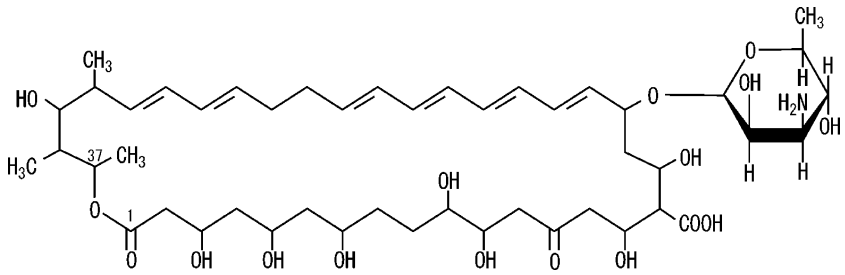

Fig. 3a Nystatin (Fungicidin)
Polyene antifungal antibiotic produced by *Streptomyces noursei, S. aureus*.

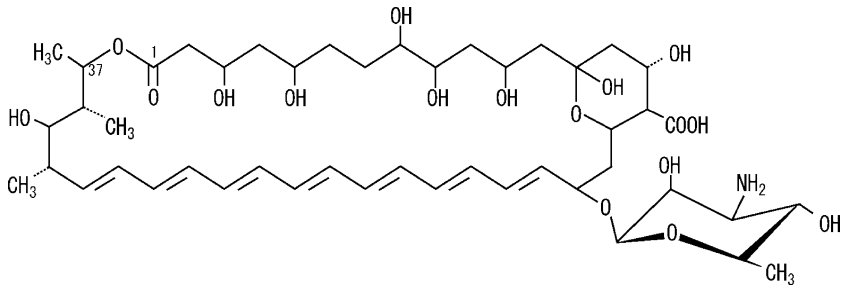

Fig. 3b Amphotericin B (Fungizone)
A polyene antibiotic produced by streptomycete culture M4575 obtained from soil of the Orinoco river region on Venezuela.

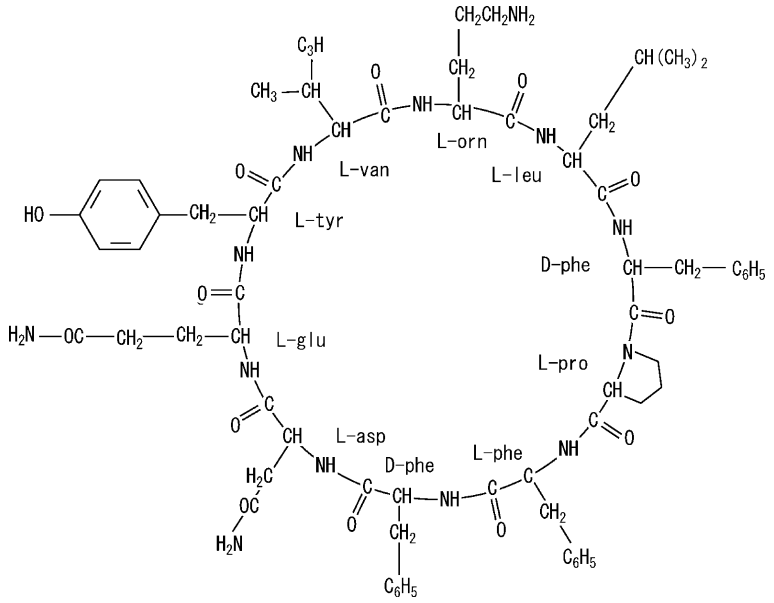

Fig. 3c Tyrocidine
Antibiotic mixture produced by *Bacillus brevis*. Major constituent of tyrothricin.

may be used to characterize the types of secondary structures present in a given protein molecule.[5] In some cases a particular amino acid sequence can be associated with a particular secondary structure, based on either theoretical[6] or experimental considerations. For example, the bacteriorhodopsin molecule is known to consist of seven transmembrane α-helical segments connected by short linker sequences.[7] Because of the protease susceptibility of some of the linker regions, and the occurrence of helix breaking residues (proline), the amino acid sequence of bacteriorhodopsin can be approximately aligned with the α-helical segments.[8] Another example is the case of gramicidin A, for which several helical conformations have been proposed.[2),9)] A particular polypeptide backbone secondary structure may impose limitations upon the availability of allowed conformations of amino acid side chains which are attached to that backbone. This paper concerns the calculation of possible side chain orientations based on steric interactions with a given polypeptide backbone structure.

The general question under consideration here is the placement of amino acid side chains in polypeptides of known sequence and known or proposed secondary structure. Although the orientations of side chains in such cases cannot be determined absolutely, the range of allowed orientations may be computed for an individual side chain attached to a given backbone structure. Information regarding the orientations of side chains may be important for considerations of protein function in membranes (for example, the proton pumping activity of bacteriorhodopsin), or for deciding between alternative proposed secondary structures. In the absence of side chains, it was impossible to determine whether a single-stranded β-helix or a double-stranded β-helix gave a better fit to the diffracted x-ray intensities from ionfree crystals of gramicidin A.[10]

This paper provides a general method for attaching side chains to a polypeptide backbone structure, once coordinates for the backbone are available. To illustrate the method, we discuss the attachment of tryptophan side chains to a single-stranded β-helical model of gramicidin A. The coordinates of the backbone structure, including β-carbons, were generated by a computer method for building dipeptide repeat helices.[11]

Gramicidin A is a linear peptide of 15 amino acids described before, including 1 Gly, 2 Ala, 4 Val, 4 Leu, and 4 Trp, the latter at positions 9, 11, 13 and 15.[12] The-Gly and Ala side chain positions are completely determined by the backbone structure, the Val side chains have one degree of freedom – rotation about the C_α-C_β bond and the Leu and Trp side chains have two degrees of freedom – rotations about the C_α-C_β and C_β-C_γ bonds. Because of the large size of the indole ring, the Trp orientations are most restricted by the helical backbone. Therefore, our attention has focused mainly on tryptophan, although the same approach can be used for any amino acid.

II. Computer fitting of tryptophan

(1) Starting backbone model

A family of single-stranded β-helical structures was proposed in 1971, and approximate helix parameters were derived from CPK and/or wire models of the helices.[13] The β-helices having 4.4, 6.3 or 8.2 residues per turn were shown to be stabilized by favorable hydrogen bond patterns

similar to those of parallel β-pleated sheet. The β-helix having 6.3 residues per turn is the most likely candidate for the conformation of the gramicidin A transmembrane channel.[1),14),15)] It has been found that a single-stranded β-helix having 6.3 residues per turn and a pitch of 4.85 forms favorable 2.8 Å intrachain hydrogen bonds, and used computer generated coordinates for this helix[11)] as a starting polypeptide backbone to which attach Trp side chains.

(2) Attachment of side chains to the helix

The side chains are attached by a least-squares best molecular fit to the x-ray crystal structures of the amino acid monomers. The method of the least-squares best molecular fit is the same as described by Nyburg[16)], except that the atomic coordinates of the nitrogen (N), alpha carbon (C_α), beta carbon (C_β), and carbonyl carbon (C(O)) atoms are used as the comparable sets of four atoms common to both the amino acid monomers and the peptide backbone.

The present author has developed the computer software to calculate the best position of the side chains of polipeptides by this method.

The method will be briefly described here. Input to the program consists of atomic coordinates of N, C_α, C_β and C(O) of both the backbone and the monomer. The nonweighted centroids of two sets of four common atomic positions, $(1/4\sum_{i=1}^{4}X_i, 1/4\sum_{i=1}^{4}Y_i, 1/4\sum_{i=1}^{4}Z_i)$ are computed from the atomic coordinates translated into an orthogonal system, and assigned as the origin for a calculation of either a constrained or unconstrained best molecular fit. The errors in individual atomic coordinates are not considered in the calculation described here.

After an initial rotation of the monomer using only the three points associated with the centroids and the positions of the first two atoms of the set, the fit is refined using three kinds of rotations, S_1, S_2 and S_3 of the monomer about the orthogonal axes (X, Y, Z) to give a best least-squares fit of X, Y, and Z coordinates. One such kind of rotation equation for the X-axis is as folliws[17)]:

$$S_1 = \begin{pmatrix} \cos\varpi_1 & -\sin\varpi_1 \\ \sin\varpi_1 & \cos\varpi_1 \end{pmatrix}$$

where

$$\varpi_1 = \tan^{-1}\{\sum_{j}^{n}(Y_{1j}X_{2j} - Y_{2j}X_{1j}) / \sum_{j}^{n}(X_{1j}X_{2j} - Y_{1j}Y_{2j})\}$$

If the change in ω_1 for each rotation (S_1, S_2 and S_3) between successive refinement cycles is less than 0.001°, the refinement is terminated. In all cases, no more than three redinement cycles have been required. An alternate method using orthogonal rotation matrices[17)] gives equivalent results, but requires more computation time.

(3) Rotation of the attached side chains

Crystallographic coordinates (X') are translated to orthogonal coordinates (X) by a translation matrix (T) defined by the cell dimensions, X = TX'. Fig. 4 shows a schematic diagram of a tryptophan side chain attached to the peptide backbone in orthogonal coordinate system. Consider the line ℓ_1 which passes through the points P_1 (X_1, Y_1, Z_1) and P_2 (X_2, Y_2, Z_2) associated with the C_α and C_β atoms of indole ring of tryptophan. The equation of ℓ_1 is:

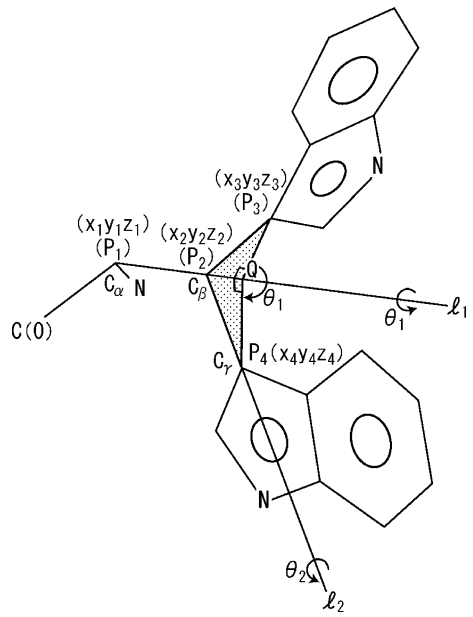

Fig. 4 Schematic diagram which shows the two rotational degrees of freedom of the indole side chain of tryptophan.
The allowed rotations are $\theta 1$ (about the Cα-Cβ bond) and $\theta 2$ (about the Cβ-Cγ bond).

$$\frac{X-X_1}{L}=\frac{Y-Y_1}{M}=\frac{Z-Z_1}{N}$$

where the direct cosines are defined by $L = X_2 - X_1$, $M = Y_2 - Y_1$ and $N = Z_2 - Z_1$. If we rotate the point P_3 through the angle θ_1 around ℓ_1, P_3 is shifted to the point P_4. The coordinates of P_4 (X_4, Y_4, Z_4) are given by the following equations:

$$X_4 = X_1 + hL + (X_3 - X_1 - hL)\cos\theta + \frac{1}{\sigma}\begin{vmatrix} M & N \\ Y_3 - Y_1 & Z_3 - Z_1 \end{vmatrix}\sin\theta$$

$$Y_4 = Y_1 + hM + (Y_3 - Y_1 - hM)\cos\theta + \frac{1}{\sigma}\begin{vmatrix} N & N \\ Z_3 - Z_1 & X_3 - X_1 \end{vmatrix}\sin\theta$$

$$Z_4 = Z_1 + hN + (Z_3 - Z_1 - hN)\cos\theta + \frac{1}{\sigma}\begin{vmatrix} L & M \\ X_3 - X_1 & Y_3 - Y_1 \end{vmatrix}\sin\theta$$

where σ and h are as follows :

$$\sigma = \sqrt{L^2 + M^2 + N^2}$$

$$h = \frac{1}{\sigma^2}\{(X_3 - X_1)L + (Y_3 - Y_1)M + (Z_3 - Z_1)N\}$$

For each rotation (θ_1) around ℓ_1 as the first rotation, a series of second rotations (θ_2) around the line ℓ_2 (defined by the points P_2 and P_3) are carried out to obtain all possible tryptophan side chain conformations. The author choses to examine the steric contacts between the side chain

and the helical backbone at intervals of 30° in θ_1 and 30° in θ_2. In this way 144 different tryptophan conformations were considered.

III. Results

Using a single-stranded, left-handed β-helix having 6.3 residues per turn and a pitch of 4.85 as the model for the polypeptide backbone of gramicidin A (Fig. 7), including β carbons, the side chain of residue 9, L-Trp, was attached to the backbone by the method described above. Stereo ORTEP drawing (Fig. 8) of an antiparallel dimer of single-stranded β-helices (P=4.85, N=6.3), which are possible structures of Gramicidin A in membrane. The tryptophan side chain was then rotated around the C_α-C_β single bond (θ_1) and the C_β-C_γ single bond (θ_2) from 0 to 360° in intervals 30° for both rotations, to give 144 possibilities for the side chain conformation. For each orientation, a forbidden van der Waals overlap test was done by calculating the shortest distance from any backbone atom to any side chain atom, to give the steric map shown in Fig. 5. In Fig. 5, the allowed positions of No. 9 tryptophan are shown in the shaded area. For all other orientations an impossible close van der Waals contact (less than 2.9 Å) occurs between the helix and the side chain. The possible conformations are roughly separated into three regions, with θ_1 in

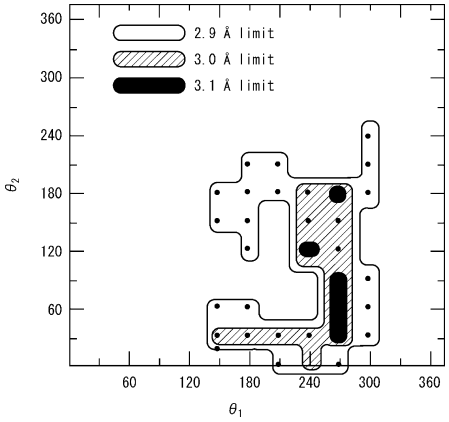

Fig. 5 Illustration of the allowed orientations of indole rings attached to odd numbered (L) α-carbons of a left-handed N = 6.3, P = 4.85 β-helix

The $\theta 1$ and $\theta 2$ rotational angles are as defined in Fig. 4. For each orientation, the distance of closest approach between the indole ring and the polypeptide backbone was calculated. The shows contours surrounding orientations whose minimum contact distances are 3.1, 3.0 or 2.9 Å. If the minimum distance was less than 2.9 Å, the orientation was disallowed. The results are the same as Trp-9, 11, 13 and 15 in the amino acid sequence of gramicidin A. All of the allowed orientations for these tryptophans direct the indole rings toward the N-terminal end of the helical backbone assumed for the gramicidin A molecule. This is true even for the C-terminal Trp-15 group, since local contacts between the C-γ carbon and neighboring atoms of residues 14, 15 and ethanolamine preclude those values of the $\theta 1$ angle which would tend to point the ring into the space off the C-terminal end of the molecule.

the range from 150°-300°, if θ_2 is near either 30° or 180° ; or θ_2 in the range from 0°-180°, if θ_1 is near 270°. The θ_1 and θ_2 values are measured relative to the conformation of L-Trp in single crystals of the pure amino acid.[18)]

For additional information on the selection of a preferred conformation, the distances (d_1) between the nitrogen atom of the backbone and the C_γ atom, and the distances (d_2) between the carbonyl carbon atom of the backbone and the C_γ atom were calculated, because the position of the gamma carbon atom of the side chain is restricted only by the θ_1 rotation independent of the second rotation (θ_2). When the distance d_2 is plotted versus d_1, the egg-shaped smooth curve shown in Fig. 6 is obtained. Ramachandran and Sasisekharan[19)] surveyed extensive experimental evidence on the structures of amino acid and peptides, and established characteristic distances between the atoms corresponding to the shortest intra- and intermolecular contacts. According to their table, normal limits of N・・・C_γ and C・・・C_γ are 2.9 and 3.0 Å, respectively, and their extreme limits of close approach are 2.8 and 2.9 Å, respectively. On the basis of their values with regard to the distances N・・・C_γ and C(O)・・・C_γ described above, the allowed positions of the C_γ atoms of tryptophan side chain are shaded in Fig. 6.

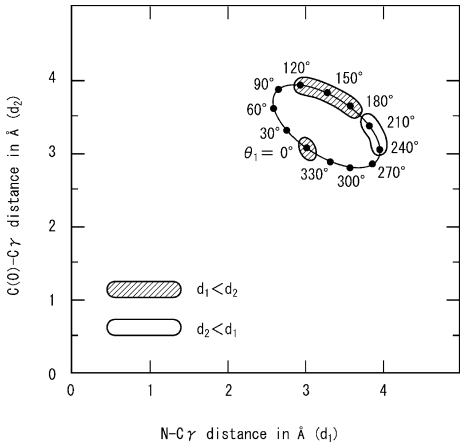

Fig. 6 Variation of Trp Cγ-to-backbone contact distances with the rotation angle θ1 (defined in Fig. 4).

By combining the information in Fig. 5 and 6, one can restrict the orientation of the side chain of Trp-9. If $d_1 < d_2$ in Fig. 6, as is generally found[19)], then θ_1 is restricted to the range 120°-180° and θ_2 is restricted to values near 30° or 180°. If $d_2 < d_1$ the allowed region is enlarged to include θ_1 values up to 240°. When the calculations are repeated for Trp-11, 13, and 15 of gramicidin A, identical steric constraints are obtained, a result which would be expected for a dipeptide repeat helix in which all oddnumbered residues of the polypeptide backbone are equivalent, provided there were no relaxing of constraints near the end of helix. The possibility of a helix termination effect with regard to Trp-15 was of special interest, since this is the final amino acid in the sequence. However, the C-terminal of Trp-15 is blocked by ethanolamine. With ethanola-

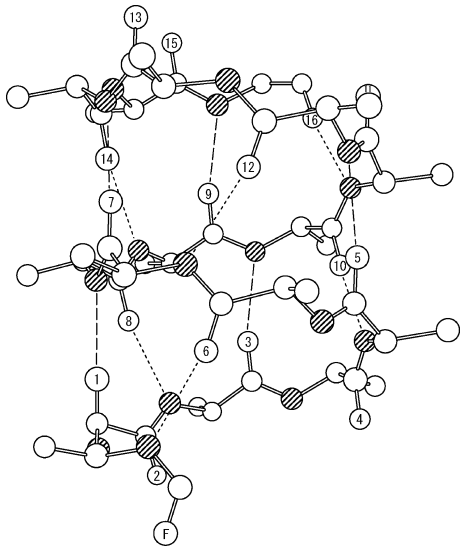

Fig. 7 ORTEP drawing of a left-handed single-stranded β-helix having P = 4.85 Å/turn and N = 6.3 residues/turn. This and subsequent figures show the requisite number of nonhydrogen atoms sufficient to represent various plausible configurations for the backbone of gramicidin A, including the formyl and ethanolamine terminals. The helix progresses upward from the N-terminal formyl group at the bottom of the figure. The nitrogen atoms are crosshatched, carbon atoms left open, formyl oxygen labeled F, and other oxygens numbered. In this model, the hydroxyl of the C-terminal ethanolamine, labeled residue 16, has been arbitrarily drawn so as to hydrogen bond to the nitrogen of residue 11. The β-carbon of residue 2 (Gly in gramicidin A) has been deleted. The intramolecular hydrogen bonds (–) in this model have lengths of 2.79 Å (even-numbered carbonyl groups) or 2.82 Å (odd carbonyls). (Ref. 4)

mine included in our backbone model and oriented so that the terminal-OH is hydrogen bonded to the N-H of residue 11[11], the side chain of Trp-15 was subject to exactly the same steric restrictions as Trp-9. This result is primarily due to the fact that local interactions in the vicinity of the $N-C_{\gamma}(C)-C_{\beta}-C_{\gamma}$ atoms require that the indole ring slant toward the N-terminal direction and away from the C-terminal, thus precluding the side chain of Trp-15 from flopping into free space off the C-terminal end of the helix. Of course, this analysis would be valid only if the regularity of the helical backbone structure were maintained to the very end of the chain, with the final intrahelix hydrogen bonds intact:

	14 C=O	····	H-N	9
ethanolamine	N-H	····	O=C	9
ethanolamine	C-O	····	H-N	11
	H			

(The carbonyls and nitrogens of residues 13 and 15 point out into solvent off the C-terminal end of the helix).

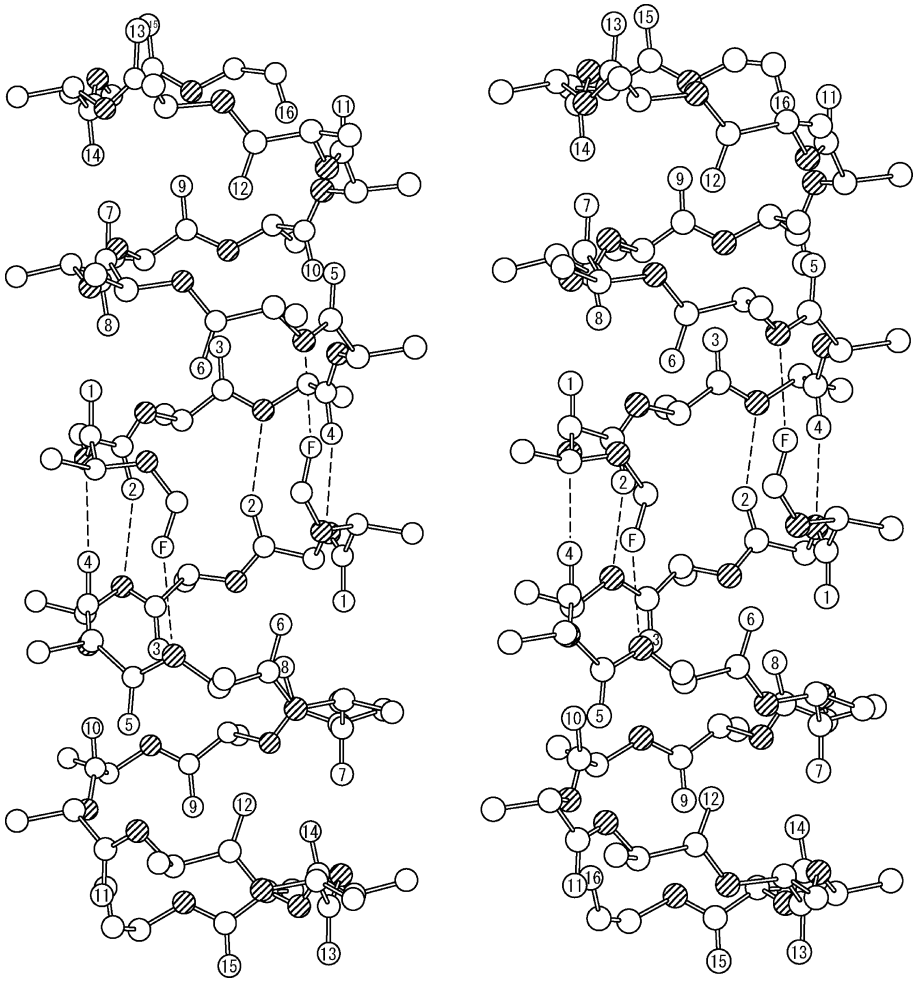

Fig. 8 Stereo ORTEP drawing of an antiparallel dimer of single-stranded β-helices (P = 4.85, N = 6.3). Each monomer has the structure shown in Fig. 7. All intramolecular hydrogen bonds have been omitted, but the six intermolecular hydrogen bonds that have been proposed to stabilize a gramicidin A dimer (Ref. 4) are shown.

Two of the sterically allowed tryptophan orientations are shown in Fig. 9. The ORTEP plotting program20) was used to display the end views of models of a gramicidin monomer having all of the Trp side chains in the (θ_1 = 240°, θ_2 = 150°) orientation (Fig. 9).

IV. Discussion

This paper presents a method for calculating the restrictions on Trp indole ring orientations imposed by a helical polypeptide backbone. Now coordinates of the Trp side chains, in one of a small number of allowed positions, can be included in a backbone model of gramicidin A. Since

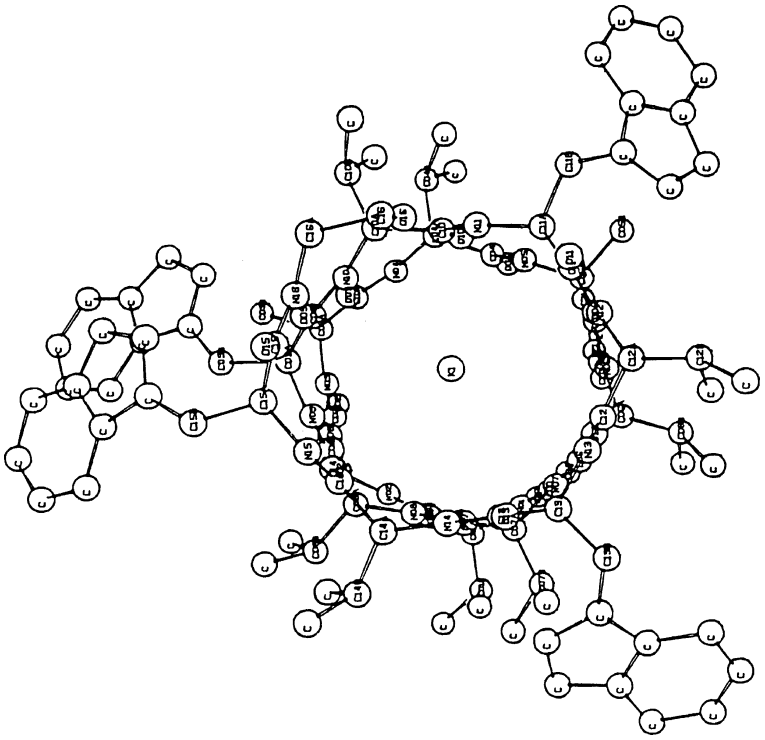

Fig. 9 End view of a β-helical model as in Fig 8, except that the Trp side chains are drawn in the ($\theta 1$ = 240°, $\theta 2$ = 150°) orientation (defined in Fig. 4, 5 and 6)

four of the fifteen amino acids in gramicidin A are Trp, the overwhelming majority of side chain electron density is included in these four side chains. Inclusion of these atoms in the model should help attempts to resolve the remaining single isomorphous phase ambiguity for crystals of ionbound gramicidin A[21], by a trial model building approach.

References

1) Weinstein S, Wallace BA, Blout ER, et al: Conformation of gramicidin A channel in phospholipid vesicles: a ^{13}C and ^{19}F nuclear magnetic resonance study. Proc Natl Acad Sci USA 76: 4230~4234, 1979.
2) Urry DW: The gramicidin A transmembrane channel. A proposed π(L, D) helix. Proc Natl Acad Sci USA 68: 672~676, 1971.
3) Wallace BA: Gramicidin channels and pores. Annu Rev Biophys Biophys Chem 19: 127~157, 1990.
4) Kimura M, et al: Computer building of β-helical polypeptide models. Biopolymer 23: 23~38, 1984.
5) Chen YH, Yang JT, Chau KH: Determination of the helix and β-form of proteins in aqueous solution by circular dichroism. Biochemistry 13: 3350~3359, 1974.
6) Chou PY, Fasman GD: Prediction of protein conformation. Biochemistry 13: 222~245, 1974.
7) Henderson R, Unwin PN: Three-dimensional model of purple membrane obtained by electron microscopy. Nature 257: 28~32, 1975.
8) Engelman DM, Henderson R, McLachlan AD, et al: Path of the polypeptide in bacteriorhodopsin. Proc

Nalt Acad Sci USA 77: 2023~2027, 1980.
9) Veatch WR, Fossel ET, Blout ER: The Conformation of gramicidin A. Biochemistry 13: 5249~5256, 1974.
10) Koeppe Ⅱ RE, Hodgson KO, Stryer L: Helical channels in crystals of gramicidin A and of a cesium-gramicidin A complex: an x-ray diffraction study. J Mol Biol 121: 41~54, 1978.
11) Low BW, Greenville-Wells HG: Generalized mathematical relationships for polypeptids chain helices. The coordinates of the π-helix. Proc Nalt Acad Sci USA 39: 785~801, 1953.
12) Sarges R, Witkop B: Gramicidin A. V. The structure of valine and isoleucine-gramicidin A. J Am Chem Soc 87: 2011~2020, 1965.
13) Urry DW: β-helical structure of polypeptide: In Conformation of Biological Molecules and Polymers. The Jerusalem Symposia on Quantum Chemistry and Biochemistry Vol.5 (ed by Bergmann ED, Pullman B), Israel Academy of Sciences and Humanities, 1973, pp 723~736.
14) Ramachandran GN, Chandrasekaran R: Conformation of polypeptide side chane. Indian J Biochem Biophys 9: 1~11, 1972.
15) Urry DW, Long MM, Jacobs M, et al: Conformation and molecular mechanisms of carriers and channels. Ann N Y Acad Sci 64: 203~220, 1975.
16) Nyburg SC: Some uses of a best molecular fit routine. Acta Crystallogr B 30: 251~253, 1974.
17) Korn GA, Korn TM: equation (14. 10-6). In: Mathematical Handbook for Scientists and Engineers, McGraw-Hill, 2nd ed, 1968, p 473.
18) Takigawa T, Ashida T, Sasada Y, et al: The crystal structures of L-tryptophan hydrochroride and hydrobromide. Bull Chem Soc Jpn 39: 2369~2378, 1966.
19) Ramachandran GN, Sasisekharan V: Conformation of polypeptides and proteins. Adv Protein Chem 23: 283~438, 1968.
20) Johnson CK: ORTEP ORNAL-3794, Oak Ridge National Laboratories, Nashville, Tennessee, 1965.
21) Koeppe Ⅱ RE, Berg JM, Hodgson KO, et al: Gramicidin A crystals contain two cation binding sites per channel. Nature 279: 723~725, 1979.

〔2008. 9. 29 受理〕